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Sickle Cell Acute Chest Syndrome Associated With Parvovirus B19 Infection: Case Series and Review

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Acute Chest Syndrome (ACS) continues to be a major source of morbidity and mortality among patients with sickle cell disease. It is characterized by the presence of pleuritic chest pain, fever, rales on lung auscultation, and pulmonary infiltrates on chest X-ray [Castro et al: *Blood* 84:643–649, 1994]. The pathophysiology of this disorder remains poorly understood leading to the descriptive term "Acute Chest Syndrome" designated by Charache et al. [*Arch Intern Med* 139:67–69, 1979]. Typical bacterial pathogens are seldom isolated in adults, although they play a significant role in the pathogenesis of this entity in children. Until recently, the technology to accurately study viral infection as a precipitating cause of ACS has been unavailable.

Parvovirus B19 is being increasingly recognized as an important human pathogen, and has been established as the cause of transient "aplastic crisis" in patients with sickle cell disease [Saarlen et al: *Blood* 67:11411–11417, 1986; Young: *Sem Hematol* 25:159–172, 1988]. We present three patients with hemoglobin SC variant of sickle cell disease who developed ACS in association with acute parvovirus B19 infection, one of which died of respiratory failure. Parvovirus B19 infection was established by polymerase chain reaction for parvovirus B19 DNA, and the presence of parvovirus B19 specific IgM antibodies. These cases suggest that parvovirus B19 may be associated with more than self-limited illness in patients with sickle cell disease, and that this ubiquitous virus may merit further study as a precipitating cause of ACS.

Key words: parvovirus B19, bone marrow, hemoglobin SC disease, hemoglobinopathies

INTRODUCTION

Acute chest syndrome (ACS) is the major cause of death in adults with sickle hemoglobinopathies and accounts for 15% of deaths in patients without other concomitant organ dysfunction [2]. The diagnosis of ACS is often ambiguous since many heterogenous pulmonary insults can result in its manifestations. The pathophysiology of ACS is poorly understood, and it is often difficult to distinguish from pneumonia. Bacterial pathogens are seldom identified in adults, despite the presenting features of fever, cough, chest pain, leukocytosis, and new infiltrates on chest X-ray [3]. Nonbacterial causes such as intrapulmonary sickling with in situ thrombosis [4], pulmonary infarction [3–7], marrow embolization [8,9], and rib-infarction [10,11] have also been implicated. The precipitating event for such alterations in homeostasis continues to be unclear. Of interest, pulmonary findings have long been appreciated as a component of aplastic crisis [12]. Likewise, a decline in hematocrit is frequently observed with ACS [7]. In this report, we provide evidence that parvovirus B19 may be associated with both entities, which may be part of an overlapping spectrum of illness in patients with sickle cell disease.

Parvovirus B19 has a predilection for infecting human erythroid progenitor cells and has been established as the primary etiologic agent of transient erythroid aplasia in patients with chronic hemolytic anemia, including sickle cell disease [1] and hereditary pyropoikilocytosis [13]. Parvovirus B19 is a ubiquitous, heat stable, DNA virus that is cytotropic to proliferating cells that express P

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antigen [14]. P antigen, or globoside, is expressed on erythrocytes, erythroblasts, megakaryocytes, and endothelial cells [15]. It has been shown to be the specific receptor for parvovirus B19, since cells lacking this receptor are immune to infection [16].

Although generally causing indolent, self-limited symptoms in the normal host (such as erythema infectiosum and post infectious arthropathy), parvovirus B19 can cause complications such as hydrops fetalis [17]. In patients with sickle cell disease, acute parvovirus B19 infection may cause symptoms ranging from those of a typical viral syndrome, to hemodynamically significant anemia, and, as related in this report, it can be associated with serious respiratory morbidity and mortality. We have documented three cases of ACS which were associated with acute Parvovirus B19 infection. This was established by the presence of an acute febrile illness associated with reticulocytopenia, anemia, parvovirus B19 DNA in serum or marrow samples, and an elevation of parvovirus B19 specific IgM antibodies. These cases are reviewed and the pathophysiologic implications are discussed.

METHODS AND MATERIALS

Three male patients with hemoglobin SC disease were admitted to the University of Alabama hospital for acute respiratory illness associated with a declining hematocrit. In patient 1, parvovirus B19 DNA was isolated from bone marrow after saponin treatment and subsequent lysis of nucleated cells. DNA was extracted using proteinase K and SDS; 100 nanograms of sample was then processed in identical fashion to that of sera. Peripheral blood sera from patients 2 and 3 was obtained and analyzed for the presence of parvovirus B19 DNA. Sera was extracted with chloroform by methods previously described [18]. All PCR studies were performed in the Diagnostic Molecular Biology laboratory at the University of Alabama at Birmingham.

Ten microliters of the aqueous phase was amplified by 40 cycles of polymerase chain reaction (94° × 15 seconds, 58° × 90 sec, 72° × 135 sec) using Parvovirus B19-specific primers (5′ primer: ATA AAT CCA TAT ACT CAT T; 3′ primer: CTA AAG TAT CCT GAC CTT G). The product was size fractionated on a 1% agarose gel and transferred to a nylon membrane. An internal oligonucleotide (A ACT CTG TAA CTT GTA C) was used to probe the blot. Electrophoresis was performed on 1.5% Seakem (FMC, Inc., ME) agarose gel and this product was stained with ethidium bromide. This technique has a >90% sensitivity and >95–98% specificity at our institution for detecting parvovirus infection. The presence of parvovirus specific antibodies was determined by Specialty Laboratories (CA) using standard enzyme immunoassay methods.

CASE REPORTS

Patient 1 (WS)

A 22-year-old African-American male with hemoglobin SC presented with a 1 week history of progressive dyspnea on exertion, intermittent chills, and fever up to 104°F. Four days prior to admission, he developed a nonproductive cough with progressively worsening right pleuritic chest pain. The patient's usual hematocrit, documented 4 weeks prior to admission, was 40%.

His exam was notable for a temperature of 102.1°F, blood pressure of 146/80, pulse of 130 beats/min, and respiratory rate of 44/min. Sclera were icteric and conjunctivae were pale. There was dullness to percussion of the inferior one half of the right hemithorax and diffuse inspiratory wheezes were noted bilaterally on chest auscultation.

Laboratory data showed a WBC count of 20,200/mm^3 with a normal differential. His hemoglobin was 5.4 g/dl, hematocrit 15%, and platelets were 71,000/mm^3. There were nucleated red cells and many sickled cells on his peripheral blood smear, however the corrected reticulocyte count was less than 1.0%. Prothrombin time was 12.6, with an international normalized ratio of 1.33, and activated partial thromboplastin time of 28. D-dimers were positive at 1:32, and fibrinogen was 614 mg/dl.

The initial arterial blood gas analysis showed a pH of 7.50, pCO_2 of 34 mmHg, pO_2 of 60 mmHg, and SAO_2 of 82%. The initial chest X-ray demonstrated an infiltrate in the right hemithorax with a moderate effusion for which diagnostic thoracentesis was performed. Pleural fluid contained no bacteria, protein was 120 g/l, glucose was 3.9 mMoles/l, and WBC count was 54,640/mm^3, with 64% segmented neutrophils, 7% lymphocytes, and 26% monocytes. A chest tube was placed for drainage. All cultures of this fluid were negative.

The patient's gas exchange improved markedly with transfusion of 2 units of packed cells and supplemental oxygen. Pancytopenia developed on the third hospital day, and a bone marrow biopsy was performed. His marrow was necrotic, and normal cellular architecture was replaced with amorphous debris (Fig. 1). The marrow was assessed for the presence of parvovirus B19 DNA by polymerase chain reaction (PCR), and this was positive (Fig. 2). Due to severe back and pelvic pain, an MRI scan was performed which showed acute avascular necrosis of both femoral heads and segmental infarction of vertebral bodies from T9-S2.

Parvovirus B19 infection was treated with intravenous gammaglobulin 35 g/d for 10 days. Within 3 days of initiation of therapy, the platelet and WBC counts had returned to normal, but the patient required an additional 6 units of packed red cells. He was discharged home on the 26th hospital day with a hemoglobin of 10.5 g/dl, WBC count of 8,000/mm^3, platelet count of 561,000/

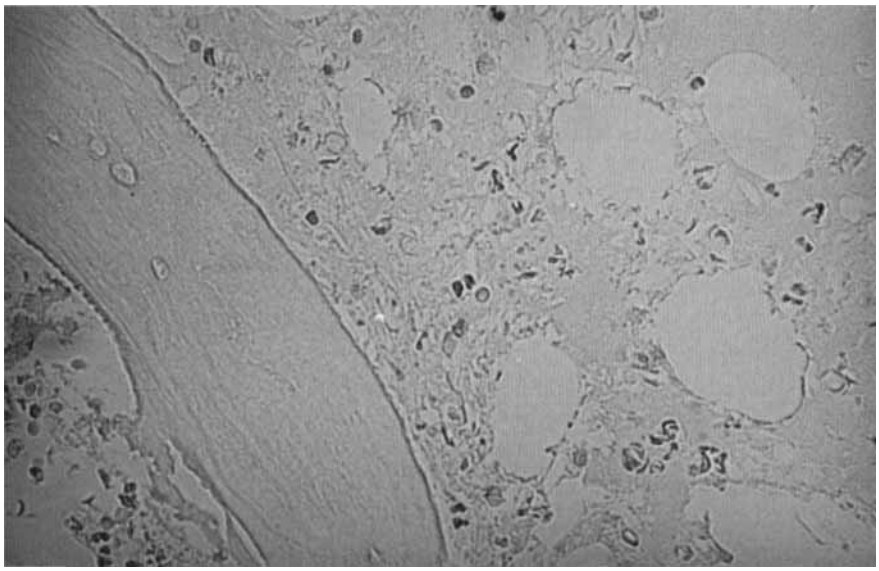

Fig. 1. Trephine biopsy from posterior iliac spine demonstrating areas of marrow necrosis with pynknotic nucleii, absent cytoplasm, and abundant amorphous substance. (Hematoxylin and eosin stain, original magnification ×50).

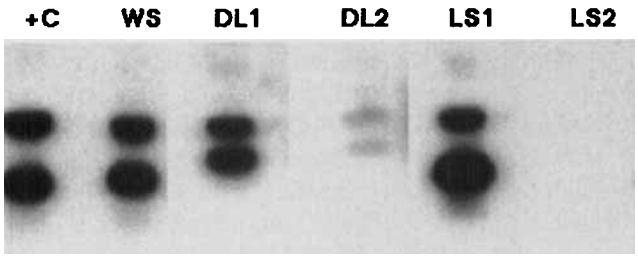

Fig. 2. Presence of Parvovirus B19 DNA in patient sera. Lanes on figure correspond to: +C, positive control (~170 virus genome equivalents); WS, patient WS on hospital day 5 at initiation of gammaglobulin; DL1 and DL2, patient DL on hospital days 2 (prior to gammaglobulin) and 7 (after 5 days of gammaglobulin therapy), respectively. This is a composite blot in which the +C and WS were run on one day and blot, and DL1 and DL2 on a different blot (development of this blot was adjusted for equivalent strength of the +C). LS1 corresponds to hospital day 4 prior to red cell exchange and gammaglobulin. LS2 demonstrates complete resolution of parvovirus B19 viremia from a serum sample obtained 1 week following treatment. This correlated with a return of his baseline reticulocyte count.

mm^3, and reticulocyte count of 8%. The right lower lung infiltrate was markedly improved at the time of discharge, and there have been no long term sequelae of this infection on 12 months of follow-up.

Patient 2 (DL)

A 27-year-old African-American male with hemoglobin SC disease, presented with a 2 day history of fever, chills, sweats, nonproductive cough, and bilateral pleuritic chest pain along with progressively worsening thigh and back pain. He was known to have a chronically enlarged spleen, usually ascertained to be 3–4 cm below the left costal margin, which was unchanged. His exam was otherwise unremarkable.

The initial chest roentgenogram showed no infiltrates. Room air blood gas showed a pH of 7.52, pCO_2 of 30 mmHg, pO_2 of 79 mmHg, and SAO_2 98%. The patient smoked one pack of cigarettes per day and had a carboxy hemoglobin of 4%, resulting in the apparent discrepancy between SAO_2 and PO_2. The initial WBC count was $3{,}800/mm^3$ with a normal differential, the hematocrit was 23%, reticulocyte count was 0.1%, and platelets were $72{,}000/mm^3$. The patient's baseline hematocrit was 41%, prior to this presentation.

The patient was admitted for routine management of his sickle crisis, and within 12 hr developed board-like abdominal rigidity, was unresponsive, and SAO_2 was unmeasurable by finger oximetry. A stat hematocrit was 9.0%. He was never hypotensive, and a packed red cell transfusion was administered as soon as compatible units could be obtained. A portable abdominal ultrasound showed echogenic kidneys with a markedly enlarged spleen extending to the pelvis, with suggestion of retroperitoneal fluid accumulation.

A splenectomy was performed, and pathologic findings were consistent with acute splenic sequestration, and there was no evidence of rupture or infarction. Owing to pancytopenia, a peripheral blood specimen was analyzed for the presence of parvovirus B19 DNA by PCR, which was detected (Fig. 2). Additionally, anti-parvovirus B19 IgM

was detected by immunoblot technique, further suggesting acute infection with this organism. The patient was treated postoperatively with 40 g of intravenous gammaglobulin daily for 5 days, along with mechanical ventilation, and transfusion to maintain a hematocrit of 25%. A second assay for parvovirus B19 DNA showed the viral DNA load to be greatly reduced (Fig. 2).

The patient's postoperative course was characterized by progressive respiratory failure with the development of bilateral patchy alveolar-interstitial pulmonary infiltrates, worse in the lower lung zones, with small bilateral pleural effusions. A hemoglobin electrophoresis obtained on his first day post-splenectomy showed a transfused hemoglobin A level of 74%, therefore, exchange transfusion was not performed. The reticulocyte count improved to 10%, and no further transfusions were required after hospital day 12. The patient developed acute renal failure requiring hemodialysis, progressive pulmonary deterioration requiring 100% oxygen and maximal ventilatory support, and pressor-refractory hypotension. He died on the 19th hospital day.

During his hospitalization, there was ongoing evidence of microangiopathic hemolysis with schistocytosis. He had persistently elevated D-dimers ranging from 1:8 to 1:25 in titer, along with an elevated fibrinogen, and low antithrombin III level. Futile attempts to correct this included administration of ATIII concentrate and fresh frozen plasma. Fat globules were noted in his peripheral blood samples on the 3rd and 4th hospital days, suggestive of fatty embolization from marrow necrosis or infarction. A right heart catheterization was performed on the 4th postoperative day and was notable for an elevated PCWP and a pulmonary artery pressure of 43 mmHg. This was thought to be consistent with acute cor pulmonale. His PA pressures remained elevated at the time of his death. A limited autopsy exam showed the lungs to be grossly hemorrhagic, boggy, and diffusely firm with no discreet consolidation to suggest pneumonia. Microscopic exam showed that extensive fibrosis had replaced normal septal architecture, and alveoli were obliterated by fibrin deposition. This was thought to be compatible with end-stage adult respiratory distress syndrome.

Patient 3 (LS)

A 21-year-old African-American male, also with hemoglobin SC disease presented to the emergency department with a 2 day history of severe low back pain that radiated to the hips. This was preceded by a febrile illness 1 week before his admission. Radiographic evaluation with plain films and a lumbar MRI was negative for abscess or bone infarct. The initial chest X-ray was unremarkable.

On the day following hospitalization, fever of 103°F developed and the hematocrit dropped from 42% to 22%. The reticulocyte count dropped from a baseline of 4% to 0.4%. A marked elevation in lactate dehydrogenase to 1,180 U/L and indirect hyperbilirubinemia occurred this same day suggesting that increased hemolysis may have possibly contributed to the decline in hematocrit. The following day, the patient developed mild pleuritic chest pain and severe hypoxia with a pO_2 of 44 mmHg. The chest X-ray demonstrated bilateral patchy air space opacities and bilateral pleural effusions. All blood, sputum, and urine cultures were negative for bacterial pathogens. Parvovirus B19 DNA was detected in a serum specimen by polymerase chain reaction (Fig. 2). Parvovirus B19 infection was also confirmed by the presence of elevated specific IgM antibodies with only a modest increase in parvovirus B19 specific IgG on enzyme immunoassay.

The patient was transferred to the medical intensive care unit and 100% oxygen was administered with a non-rebreather mask. Euvolemic red cell exchange was performed for suspected acute chest syndrome. One dose of intravenous gammaglobulin was also administered. The patient recovered well and was discharged on the 10th hospital day with a reticulocyte count of 6.9% and a hematocrit of 24%.

DISCUSSION

The etiology of ACS in adults remains unclear. Vichinsky et al. have recently emphasized the importance of pulmonary fat embolism (PFE) in the pathophysiology of ACS with the demonstration that PFE occurs in 44% of sickle cell patients presenting with ACS [8]. Bronchoalveolar lavage for lipid laden macrophages is diagnostic of fat embolism. Spirometry has recently been demonstrated to reduce pulmonary complications in the majority of patients with sickle cell disease, especially in the 39.5% that present with thoracic bone infarctions and resultant atelectasis [11]. However, the factors precipitating marrow fat embolization, and rib infarctions in adults with sickle cell disease remain undefined.

The association of parvovirus B19 with ACS raises several theoretical issues worthy of consideration. Enhanced vascular adhesion has been demonstrated with endothelial cells infected with herpes simplex virus type I, which increased sickle erythrocyte adhesiveness 2–4-fold [19]. The overall severity of sickling disorders seems to correlate directly with the propensity of sickle erythrocytes to adhere to vascular endothelium [20]. Recently, chronic infection with parvovirus B19 has been suggested as a cause of systemic necrotizing vasculitis [21] in patients without intrinsic immune deficiency. With the knowledge that endothelial cells express p antigen, the receptor for parvovirus B19, it seems possible that endothelial injury could be induced by binding of this virus. Increased adherence of sickled erythrocytes with activation of the coagulation cascade and subsequent vascular occlusion could result in end-organ infarction (Fig. 3).

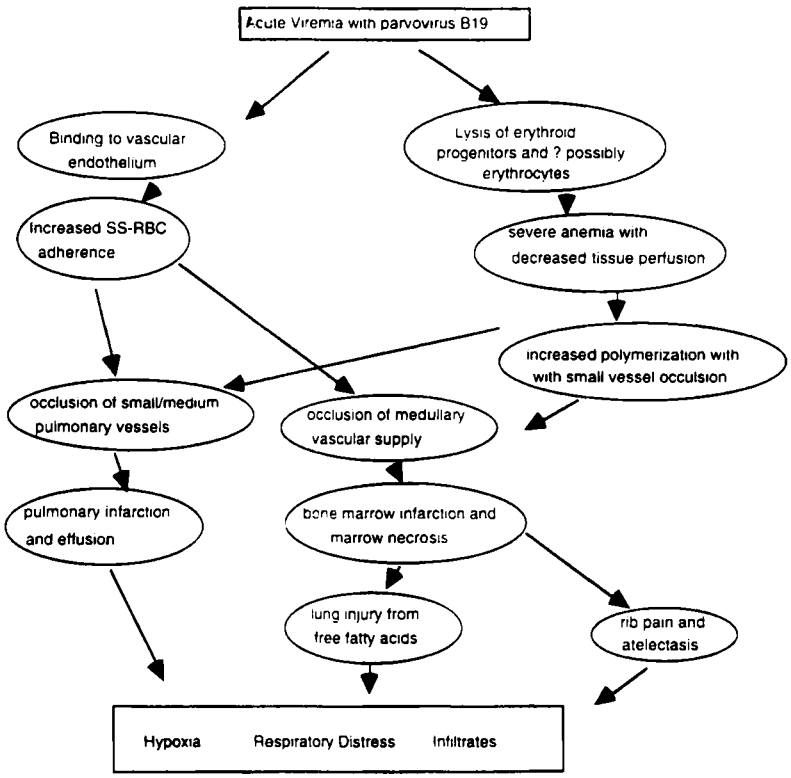

Fig. 3. Potential mechanisms of parvovirus B19 mediated lung injury.

Parvovirus B19 induced endothelial injury could be an explanation for both marrow necrosis with fat embolization, bone infarction, and the pulmonary microvascular infarction thought to be important in the pathophysiology of acute chest syndrome. Alternatively, marrow necrosis and in situ pulmonary vascular thrombosis may have been precipitated simply by erythroid aplasia and increased hemolysis with severe anemia resulting in tissue hypoxia and a subsequent increase in polymerized erythrocytes which could theoretically induce obstruction of microvasculature without a direct viral-endothelial interaction (Fig. 3).

All of the patients in this report had hemoglobin SC disease, which is usually correlated with a higher hematocrit (30–40%), increased blood viscosity, and a resultant greater propensity for pulmonary vascular occlusion [22]. Fat embolization from marrow necrosis seems to be a more frequent complication of SC disease than of SS [9]. The medullary cavity of bone has a very limited collateral blood supply, particularly in the femoral head, sacrum, and vertebral bodies [23,24]. Theoretically, marrow necrosis could result if this fragile vascular network sustained significant endothelial injury, or in situ thrombosis.

Parvovirus B19 has been implicated as a cause of marrow necrosis in patients with sickle cell disease by other investigators [25,26]. The evidence for parvovirus B19 in these cases was based upon the presence of circulating antibodies which can be an incidental finding indicative of previous exposure. Although parvovirus B19 is directly cytotropic to specific cells within the marrow, the complete loss of architecture and stromal elements seen in Figure 1 cannot be explained by direct viral cytopathic effect, and may in fact, be related to toxic metabolites from infected normoblasts, or to proximal vascular insult. This is the first report, to our knowledge, of acute chest syndrome with marrow necrosis occurring with parvovirus B19 viremia as established with direct demonstration of circulating parvovirus B19 DNA by PCR analysis. A recent, preliminary report from another group appears to confirm these findings in one patient who had massive marrow necrosis and fat embolization associated with Parvovirus B19 infection confirmed by PCR, at autopsy [27]. PCR analysis is the most specific means of diagnosis [28,29], since populations most susceptible to persistent infection may not actually produce adequate levels of IgG and IgM antibodies for detection [30–32].

Intravenous gammaglobulin has been administered to patients with prolonged erythroid aplasia [33,34], anemia in patients with parvovirus B19 and HIV [31], and other conditions in which a defined immune deficiency was thought to preclude adequate antibody formation. Sickle cell patients in general may possess subtle abnormalities in immune function. The immune response to parvovirus B19 infection in this population seems somewhat atypical

TABLE I. Treatment Modalities Used in ACS

Supplemental oxygen
Simple transfusion
Spirometry to prevent atelectasis
? Hydration
Exchange transfusion
?Antibiotics
?Intravenous gammaglobulin

given the lack of immune-complex mediated phenomena such as arthralgias and rash usually associated with parvovirus infection. Our patients received intravenous gammaglobulin due to their severe, life-threatening illness. It is unknown as to whether there is a definite quantitative or qualitative defect in parvovirus B19 antibody production in this population. In our patients, relative viral load was estimated (data not shown), and its decrease correlated with administration of passive immunization by intravenous gammaglobulin (Fig. 2: DL1 vs. DL2 and LS1 vs. LS2). Whether this intervention has the capacity to alter the outcome, or decrease the morbidity of such clinical presentations remains to be seen. If intravenous gammaglobulin were demonstrated to decrease the duration of viremia and lower transfusion requirements, this could be of benefit to this population at risk for red cell alloimmunization. Other treatment modalities used in ACS are listed in Table I.

As molecular techniques continue to evolve, our knowledge of the events triggering acute chest, vasocclusive episodes, and acute multiorgan failure [35] will hopefully provide avenues for more specific diagnosis, prevention, and treatment of these serious complications of sickle cell disease. The role of parvovirus B19, and other viruses which may potentially interact with vascular endothelium, remains to be further delineated in terms of their ability to contribute to the vascular occlusive events implied by the presence of marrow embolization and rib infarctions. The diagnosis of parvovirus B19 infection can be readily established by polymerase chain reaction determination of parvovirus B19 DNA and parvovirus B19 specific IgM antibodies. These cases suggest a need for further study to determine the role of parvovirus B19 in the causation of acute chest syndrome. Additionally, the association of parvovirus B19 with serious complications in patients with sickle cell disease creates a rationale for the development of vaccine strategies for prevention of such complications.

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